SPACE SCIENCES LABORATORY

The Fourteenth Semiannual Progress Report

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ENZYME ACTIVITY IN TERRESTRIAL SOIL IN RELATION TO EXPLORATION OF THE MARTIAN SURFACE

Professor A. D. McLaren

1 July 1971

Department of Soils and Plant Nutrition

Space Sciences Laboratory Series 12, Issue 57

UNIVERSITY OF CALIFORNIA, BERKELEY

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ENZYME ACTIVITY IN TERRESTRIAL SOIL IN RELATION TO EXPLORATION OF THE MARTIAN SURFACE

By: W. H. Brams
R. G. Burns
A. D. McLaren (Principal Investigator)
A. H. Pukite

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PREFACE

Our objectives are to explore enzyme activities in soil, including abundance, persistence and localization of these activities, and to develop procedures for detection and assay of enzymes in soils suitable for presumptive tests for life in planetary soils.

Thus far we have developed a sensitive test for soil urease, based on hydrolysis of heat-stable ^{14}C-urea and have described the urease activity of ancient and buried soils.

We have also explored in a general way the behavior of enzymes in non-classical systems, e.g. on surfaces, in gels and coacervates, and at low humidity, as an aid to understanding enzyme action in heterogeneous systems such as in soils.

A mathematical model has been developed, based on enzyme action and microbial growth in soil, for rates of oxidation of nitrogen as nitrogen compounds are moved downward by water flow. This bio-geo-chemical model should be applicable to any percolating system, with suitable modification for special features, such as oxygen concentrations, types of hydrodynamic flow, etc.

We have developed a suitable extraction procedure for soil enzymes and have been measuring activities in one such extract in order to study how urease is complexed in soil organic matter. Nearly 30 percent of soil enzymes can be isolated as colloidal, clay-free suspensions.

PROGRESS IN RESEARCH

I. THE EXTRACTION OF UREASE ACTIVE ORGANIC MATTER FROM SOIL

The extraction techniques, as outlined previously (1, 2) have been somewhat modified. With some reliability we may now extract approximately 30% of both the organic matter and the urease activity from Dublin soil.

Summary of Procedure

1. Sonication* of a 1:10 aqueous soil suspension for 20 minutes.

2. The soil is then repeatedly extracted with solutions of salts of decreasing molarity and reactions changing from slightly acid to neutral.

3. The soil suspension is centrifuged at 18,000 g for 30 minutes and the supernatant passed through a Mandler Bacteriological Filter.**

4. The extracts were dialysed under running tap water for three days and then against distilled water for one day.

5. The precipitate formed is collected and dialysed for 24 hours against 0.1 M sodium phosphate (pH 7.0). The phosphate is changed three times during this period.

The viscous brown to black material obtained has a tendency to gel on standing. It is hereafter referred to as the "dark precipitate."

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* Circo 60 watt ultrasonic generator.

** Mandler Diatomaceous Filter Cylinder, No. 3, 5" x 1", Allen Filter Co., Toledo, Ohio.

Details of the Procedure

Sonication. Twenty-five g of soil is suspended in 250 ml of distilled water, in a 500 ml conical flask, and sonicated for 20 minutes. The flask is submerged until the surface of soil suspension comes to about 1 cm below the water level in the sonication bath.

Extraction. Three extraction procedures have been examined. Concerning the extraction of an urease active fraction, their efficiency is I < II > III.

Procedure I

	Amount, g	Approximate molarity in 250 ml volume
EDTA	0.91	0.0125
Urea	30.0	2.0
NaCl	58.5	4.0
$NaH_2PO_4 \cdot H_2O$	2.0	0.25
Na_2HPO_4	7.0	0.25

The dry salts are thoroughly mixed together and slowly added to the sonicated soil suspension, while stirring the latter constantly. When the salts are dissolved, the pH of the suspension is adjusted in the range 6.4 to 6.6 and, if necessary, 1 ml of toluene is added to the suspension and the mixture shaken for 1-2 hours in a cold room (10°C). The sedimented soil is then extracted 4 times more: once with 0.25 M Na phosphate pH 7.0, and

three times with 0.05 M Na phosphate at pH 7.0. The shaking time required is only 30 minutes for each extraction (compared to four hours as previously reported (1)).

Procedure II

	Amount, g	Approximate molarity in 250 ml volume
Na citrate	69.9	0.95
$NaH_2PO_4 \cdot H_2O$	1.75	0.05
Glycine	0.94	0.05
NaCl	29.3	2.0

The dry salts are added to the sonicated soil suspension in the same manner as described in procedure I. The pH is adjusted to 6.2 - 6.4 and, as previously, 1 ml of toluene is added if necessary. The remainder of the extraction is as described in procedure I with the following changes: the pH of the Na phosphate solutions is 6.5, and all are 0.01 M in glycine.

Procedure III

	Amount, g	Approximate molarity in 250 ml volume
EDTA	0.91	0.0125
Na_2HPO_4	24.8	0.7
$NaH_2PO_4 \cdot H_2O$	10.4	0.3
Glycine	0.94	0.05
NaCl	29.3	2.0

Excepting the salts used for first extract, the method is identical to that described in Procedure II.

Dialysis. The first extract is always dialyzed separately from the others which are combined and dialyzed together. A thick-walled, strong dialysis bag (tubing) must be used for first extract, as it swells considerably before the salts can escape. Only a small amount of precipitate forms when procedures I or III are used, and none at all with procedure II. Nevertheless, the dialyzed clear first extract of procedure II shows urease activity. The enzyme protein can be precipitated out with $(NH_4)_2SO_4$ at 0.7 saturation and collected on/in a small celite pad over #42 Whatman filter paper. Washing the pad with buffers at pH 6.15, 7.1, and 8.0 failed to extract any urease activity. Finally the celite pad was dialyzed and the suspension showed some 85% of the total urease activity that was in the first extract before $(NH_4)_2SO_4$ precipitation.

II. UREASE ACTIVITY OF INORGANIC SOIL FRACTIONS

A. Comparison of Sand with Combined Silt/Clay Fraction, No Sonication.

Five g of dry soil was added to 100 ml water in a graduated cylinder and allowed to stand for 40 seconds. The sedimented material was considered as the sand fraction; the remainder, left in suspension, was considered as the combined silt and clay fraction.

Results.

Fraction	Activity in μmoles NH_3/g/hour	
Soil	18.86	
"Sand"	14.96	18.16
"Silt/Clay"	3.20	

It can be seen that most of the activity is associated with the "sand" fraction, contrary to what was expected. It is probable that the "silt/clay" fraction is not dispersed properly and settles out with the sand fraction taking much of the enzyme activity with it.

B. Comparison of Sand with Combined Silt/Clay Fraction, Sonicated.

The method is outlined in the flow diagram, Fig. 1.

Results.

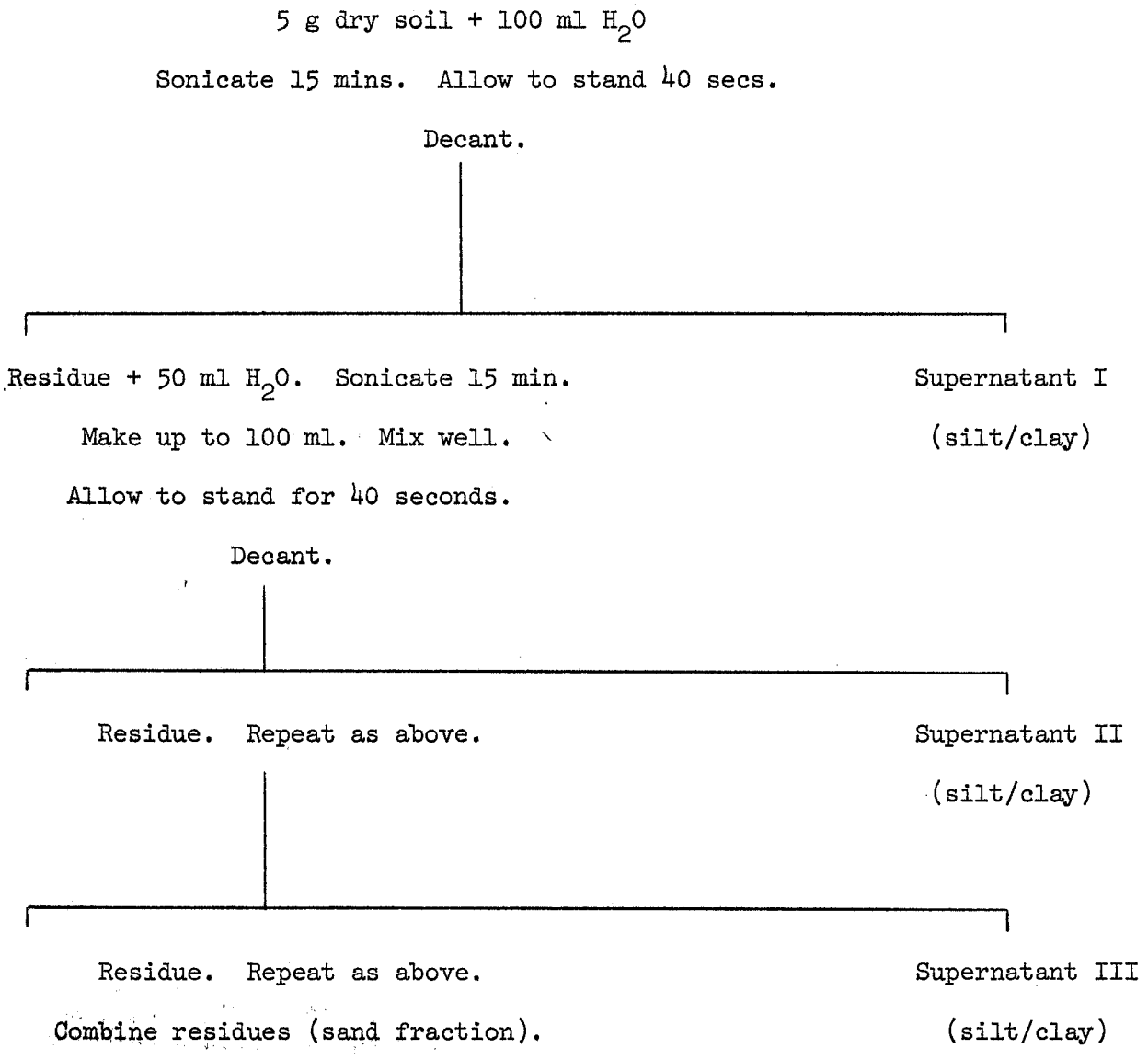
Fraction	Activity in μmoles NH_3/g/hour	
Soil	18.80	
Combined residues (sand)	0.80	
Supernatant I (clay/silt)	16.64	20.73
Supernatant II (clay/silt)	2.02	
Supernatant III (clay/silt)	1.27	

Physical separations of this nature clearly indicate that the major portion of urease activity is associated with the clay/silt fraction whilst very little is associated with the sand. To achieve this demarcation it is necessary to disperse the soil using sonication.

C. Urease Activity of Separated Clay and Silt Fractions.

The combined silt/clay fraction from experiment B above was made up to 1000 ml with water. The suspension was mixed well and allowed to settle for 2 hours at room temperature. Upon decanting, the residue is the silt fraction and the supernatant the clay.

Fig. 1. Procedure for Separation of Soil Inorganic Fractions.



Results.

Fraction	Activity in μmoles NH_3/g/hour	
Soil	18.80	
Clay	35.38	
Silt	4.96	41.14
Sand (from B)	0.80	

This further separation shows the vast proportion of urease activity associated with the clay fraction. However total activity is increased considerably (18.80 to 41.14) with this further separation. It is suggested that the extraction procedure (especially the sonication) both releases enzymes that may be unavailable in the whole soil and that the possibility of enzyme-substrate reaction is greatly increased when the large surface area of the clay fraction is exposed.

That most of the enzyme activity is associated with the clay is consistent to our idea of an organo-mineral (organo-clay) complex being the site of activity. The larger soil inorganic fractions are also associated to some degree with organic matter and would be expected to show some enzyme activity.

This experiment was repeated and the results again show a significant increase in urease activity upon separation of the clay fraction.

Results.

Fraction	Activity in μmoles NH_3/g/hour	
Soil	21.68	
Clay	53.09	
Silt	9.77	64.58
Sand	1.72	

III. EFFECT OF SONICATION ON UREASE ACTIVITY OF SOIL

The urease activity of sonicated and non-sonicated Dublin soil was tested by the Conway diffusion method (3, 4) and ^{14}C-urea methods (5) at pH 7.0 and pH 5.5, respectively.

Method	Average urease activity of Dublin soil	
	Non-sonicated	Sonicated
^{14}C-urea at pH 5.5	0.22 μM CO_2/g/hr	0.12 μM CO_2/g/hr
Conway at pH 7.0	1) 18 μM NH_3/g/hr	16 μM NH_3/g/hr
	2) 11.52 μM NH_3/g/hr	13.44 μM NH_3/g/hr
	3) 9.28 μM NH_3/g/hr	20.80 μM NH_3/g/hr

The increase or decrease in activity largely depends on intensity and length of time of sonication. Urease activity of soil seems to be less affected by sonication when measured at pH 7.0 than it is when measured at pH 5.5. Using the ^{14}C method the soil which is sonicated and dried retains more urease activity than that which is non-sonicated and dried, 0.10 µM CO_2/g/hr and 0.06 µM CO_2/g/hr, respectively.

Despite the probable loss of urease activity, "moderate" sonication of soil suspension helps to extract more organic matter and more urease activity. Also shaking-extraction times can be shortened considerably.

IV. THE RESISTENCE OF AN ORGANIC MATTER EXTRACT TO PRONASE

Using standard techniques an organic matter extract was subjected to pronase attack for six hours at room temperature, in an attempt to discover if the organic matter afforded protection to the urease enzyme.

Results.

Treatment	Activity in µmoles NH_3/ml/hr	
Organic Matter + Urea	11.67	13.52
Organic Matter + Pronase	1.85	
Organic Matter + Pronase + Urea	14.95	

These results show that there is no depression of urease activity in pronase treated extracts.

V. THE EFFECT OF TEMPERATURE ON UREASE ACTIVITY OF MOIST SOIL KEPT FOR 24 HRS AT TEMPERATURES FROM 25° TO 100°, IN 5° STEPS

This was a preliminary experiment to determine if the enzyme-organo-complex protected urease from thermal destruction.

Under these conditions urease activity of Dublin soil is lost rapidly between 35° and 55° and is reduced to zero at 60° (Fig. 2).

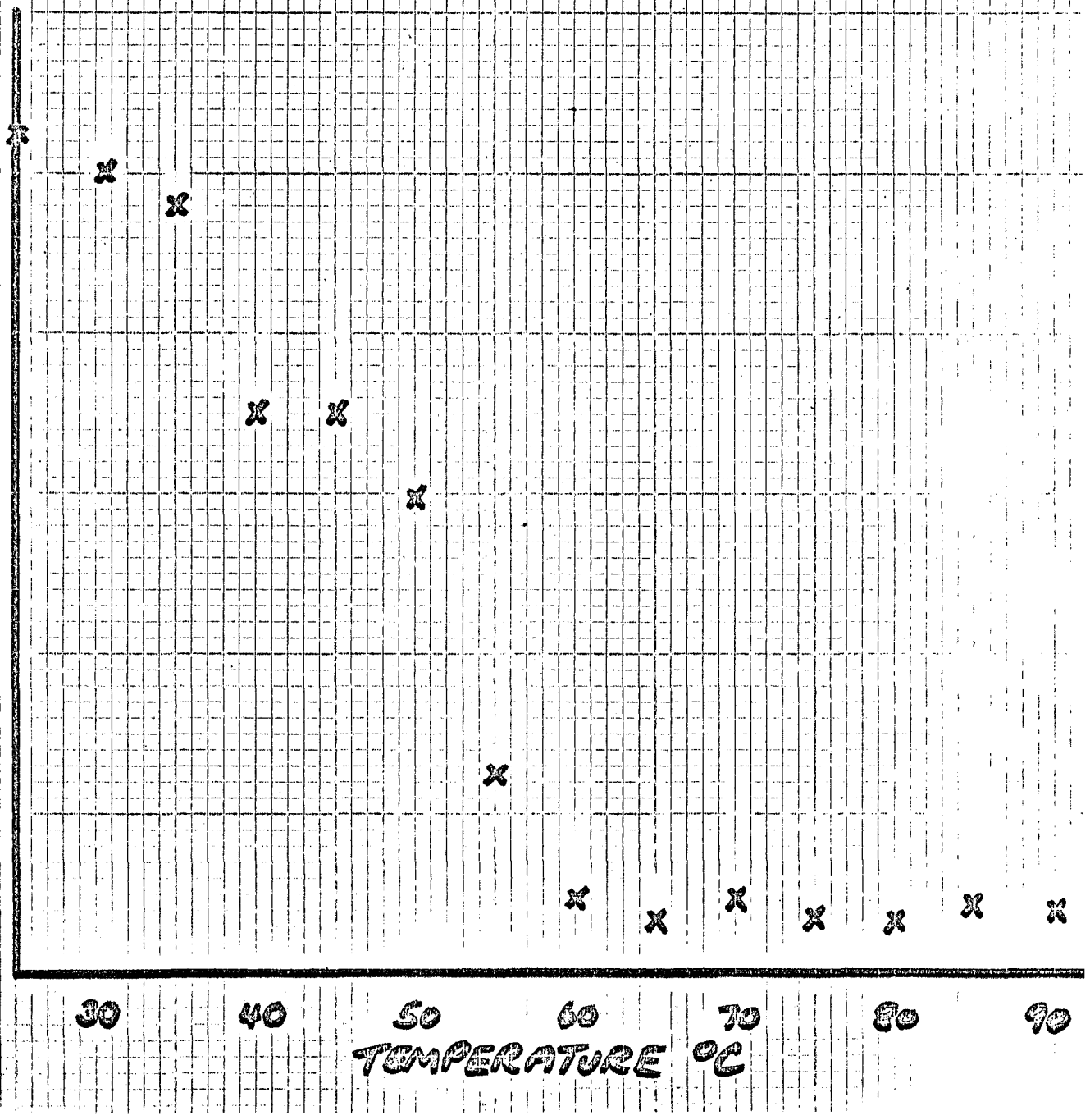
VI. EXAMINATION OF THE "DARK PRECIPITATE"

Obviously there is only a very small amount of urease activity in the dark precipitate. Attempts were made to concentrate that activity.

One M Na phosphate (pH 6.0) and Na phosphate-citrate (pH 6.5) effectively removed most of the dark colored organic fraction with very little loss of urease activity. Phosphate or citrate solution was mixed with the dark precipitate (5:1), stirred for 5-10 minutes and centrifuged at 20,000 g for 30 minutes. The dark colored supernatant was decanted. Urease activity remained in the sediment that was suspended in 0.1 M or 0.02 M Na phosphate solution at pH 7.0. Sediment can be washed further using alternately 1.0 M, pH 6.0 or pH 6.5, and 0.02 M or 0.25 M, pH 7.0, Na phosphate solutions.

Progressively less sediment remained, becoming gray-white in color, and had a high urease activity although part of whole urease activity was lost.

Fig. 2. UREASE ACTIVITY OF DUBLIN SOIL EXPOSED TO TEMPERATURES FROM 25°C TO 100°C FOR 24 HOURS.



N-N-Dimethylformamide-4 volumes of $HCON(Me)_2$ was mixed with 3 volumes of the "dark precipitate" and one volume of 1.0 M Na phosphate at pH 7.8 (0.1 M in glycine). A considerable amount of the dark organic matter was removed together with supernatant, but the urease activity of the sediment was reduced some 68%.

NH_4^+ salt solutions did not release any urease activity from dark precipitate. However, solution

1.0 mM in EDTA
0.5 M in ammonium chloride
0.5 M in ammonium citrate
+ NH_4OH conc. to pH 6.3

followed by solution (6)

0.02 M in Na phosphate
1.0 mM in EDTA
1.0 mM in mercaptoethanol

released large amounts of dark organic matter from the precipitate. Repeated washing of the sediment reduced it to about 1/10 of original volume, with little loss of activity. The color of the sediment was gray-white by the end of these manipulations.

Centrifuging suspensions of dark precipitate at 18,000 g for 30 minutes was enough to obtain clear supernatants that often possessed urease activity. When stored for several days in the refrigerator, a thin layer of sediment

appeared. If these transparent supernatants were passed through Millipore filters immediately after centrifugation, about 1/5 of the activity passes through 0.8 μ pore filter, only traces through a 0.45 μ pore filter, and none at all through a 0.22 μ pore filter.

A DEAE column, equilibrated with 0.01 M Na phosphate at pH 7.2 retains all urease activity from solutions of enzyme protein. Increasing the molarity of eluting Na phosphate to 0.2 M and the pH to 7.5 did not release any urease activity. Only a small amount (4%) of activity is eluted by 0.1 M Na phosphate at pH 7.8. The use of citrate-containing eluants has not been tried.

VII. RELATION BETWEEN UREASE ACTIVITY AND NUMBER OF BACTERIA IN "DARK PRECIPITATES"

Bacterial plate counts with 2 different media (soil extract and nutrient phosphate) showed a wide range of numbers of bacteria present in the "dark precipitate:" from 8.9×10^8 to 2.55×10^9 per milliliter. The higher counts were obtained on the nutrient phosphate agar. A gray-white material contained only 5×10^7 bacteria per ml.

Relating urease activity (in μM urea/ml/hr x 10^9) of a "dark precipitate" to the number of bacteria in one milliliter of the same, the following coeficients were obtained:

0.4

8.0

176.8

320.0

285.1

0.2

It is concluded that there is no direct relationship between enzyme activity and microbial numbers. However, the inaccuracies imposed by performing the organic matter extraction under non-sterile conditions warrants the repetition of the experiment.

It was observed, however, that a "dark precipitate," containing a low number of bacteria, had lost 50% of its urease activity after 3 months of refrigeration. A second "dark precipitate" with high numbers of bacteria in it had lost some 75% of its activity in about the same time.

VIII. SUMMARY AND CONCLUSIONS

Previous work (1) has described the extraction of an enzyme-active soil organic matter fraction from a Dublin loam soil.

It was shown that the proteolytic enzyme mixture pronase failed to effect soil urease activity whilst drastically inhibiting jackbean urease.

Bentonite-lignin complexes were prepared showing that urease may be associated with amorphous soil organic matter and that this association affords protection to the enzyme from proteolysis. The type of protection

envisaged was of a physical nature allowing for the diffusion of substrate to and product from the enzyme, but not permitting the approach of other enzymes. The comparative molecular size of enzyme, substrate and product lends itself to this hypothesis.

Work presently underway and described herein has attempted to improve both the method of extraction and the yield of enzyme-active organic matter from soil. Furthermore, experiments have been initiated which may help to illucidate more fully the physical and chemical nature of the enzyme-organic matter complex.

From the procedure described in the first section of this chapter it is apparent that sonication now plays an integral part in the extraction of enzyme-active organic matter from soil. Whilst the effects of sonication on whole soil enzyme activity are, at present, inconsistent (Expt. III), they do, however, allow a more rapid extraction of the enzyme-organic matter fraction than previously described (1).

The inconsistent effect of sonication upon urease activity presents some problems. In the first instance, sonication disrupts the soil aggregates, increasing surface area and consequently releasing previously unavailable enzyme sites. Clearly, if the enzymes are relocated on the exterior of a soil colloid there is a high potential activity. When the soil colloids are aggregated prior to sonication this potential is somewhat limited. Unfortunately the disruption effect may proceed further so that some enzymes may be destroyed by sonication. In consequence it appears that sonication, at least as far as enzyme activity is concerned, has dual

and opposite effects. Further investigations, varying length and intensity of sonication treatments, may throw some light upon these apparently conflicting results.

Experiment IV, in which an enzyme-active organic matter extract is subjected to the enzyme mixture pronase, indicates that the organic matter association protects the urease from protoeolysis. The importance of this experiment lies in the fact that no clay is present in the extract and thus clay adsorption is not, in this instance, responsible for the protection of soil enzymes. In the soil amorphous organic matter and clays are usually intimately associated due to adsorption (the so-called organo-mineral complex) and it is easy to see why the idea of clay adsorption protecting enzyme activity has been erroneously proposed and supported on many occasions.

Work was initiated to determine the size of the enzyme-active fraction and to see if the enzyme and organic matter could be separated without inhibiting activity. It is along these and other lines that future research will be directed.

BIBLIOGRAPHY

1. McLaren, A. D. (1971) Enzyme Activity in Terrestrial Soil in Relation to Exploration of the Martian Surface. Space Sci. Lab. Series 12, Issue 2.

2. Burns, R. G., El Sayed, M. H., and McLaren, A. D. (1971) Extraction of an Urease Active Organic Matter Fraction from Soil. Soil Biol. Biochem. 3(in press).

3. Obrink, K. J. (1955) A Modified Conway Unit for Microdiffusion Analysis. Biochem. J. 59:134-136.

4. McLaren, A. D., Reshetko, L., and Huber, W. (1957) Sterilization of Soil by Irradiation with an Electron Beam and Some Observations on Soil Enzyme Activity. Soil Sci. 83:497-502.

5. Skujins, J. J., and McLaren, A. D. (1969) Assay of Urease Activity Using ^{14}C-Urea in Stored, Geologically Preserved and in Irradiated Soils. Soil Biol. Biochem. 1:89-99.

6. Blakeley, R. L., Webb, E. C., and Zerner, B. (1969) Jack bean urease. A new purification and reliable assay. Biochem. 8:1984-1990.

PROGRESS IN RESEARCH

PHOSPHATASE ACTIVITY OF DUBLIN SOIL

This report continues an investigation of the characteristics of phosphatase activity of Dublin soil. Columns of soil crumbs were perfused with solutions of substrate and the relationship of the rate of the phosphatase reaction to the substrate concentration and to the height of the soil columns was determined. The activity of the soil crumbs in a non-perfused reaction system was also measured. The characteristics of phosphatase activity of the soil in these and other systems is discussed.

Materials and methods

Soil columns

The columns of soil crumbs were prepared as before (1) except that the columns were prepared in the buffered substrate solution which was to be perfused through the column. During the preparation of the columns and during perfusion the temperature of the columns was maintained at 25.0 ^{o}C by a temperature-controlled water jacket.

Phosphatase activity of soil crumbs in a stationary system

Into 50 ml Erlenmeyer flasks were placed 1.0 gm soil crumbs, 10 ml of 0.016 M sodium maleate buffer, pH 6.90, 1.6 - 48 micromoles substrate, and distilled water to 16 ml. The substrate was added last to start the reaction.

The flasks were incubated without shaking in a water bath at 25.0 °C for the indicated length of time. The flasks were swirled briefly at twenty-minute intervals. The pH of the reaction mixtures was 6.90. The reaction was stopped by the addition of 4.0 ml of 0.5 M NaOH. The solution was centrifuged and the amount of product was determined from the supernatants as before (1).
Two controls were used: flasks to which no substrate was added, and flasks to which no crumbs were added.
All flasks were prepared in duplicate and results are expressed as the average of the duplicate flasks.

Other materials and methods as before (1,3).

Results

A. Hydrolysis of buffered solutions of para-nitrophenol phosphate by columns of soil crumbs

Columns of crumbs prepared from New Dublin soil were perfused with buffered solutions of substrate and the hydrolysis of the substrate as a function of the substrate concentration was determined.

The soil columns contained 20 grams of crumbs and measured 2.1 cm in diameter by 9.0 cm high. The void volume was 24.4 cm^3. The columns were perfused with solutions of substrate in 0.01 M pH 6.90 sodium maleate buffer. The concentration of substrate in the buffer varied from 1.00×10^{-4} M to 3.00×10^{-3} M. The columns were perfused at a rate of 0.901 ± 0.033 ml/min. This rate corresponds to a solution velocity of 0.332 cm/min through the soil column and to a reaction time of 27.1 min*. Two columns were perfused for each value of substrate concentration used. During perfusion the temperature of the columns was maintained at 25.0 °C. The results of the experiment are shown in Figure 1.

The data in Figure 1 show that the characteristics of phosphatase activity of the column of crumbs were similar to those of previous columns perfused at different

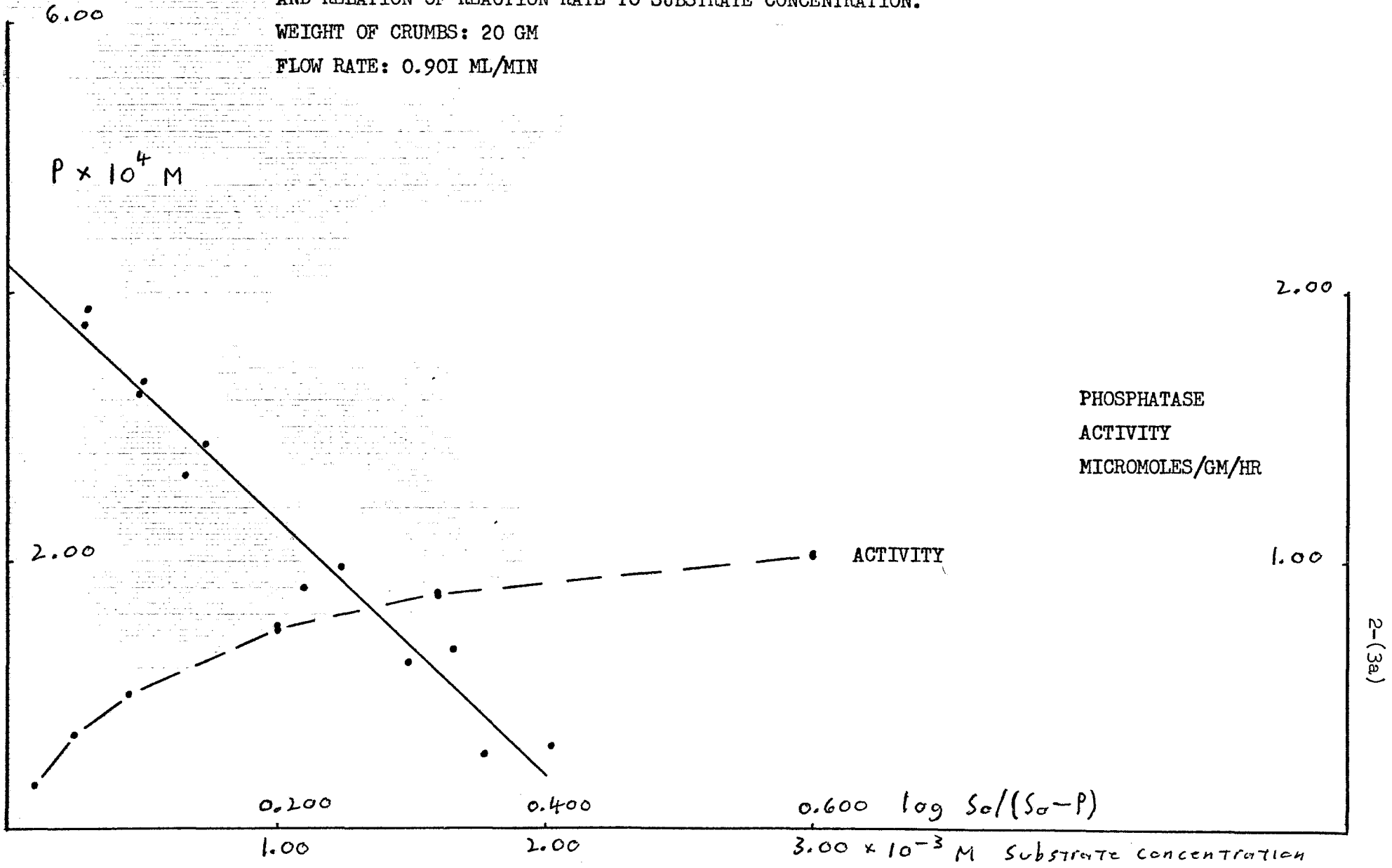
* reaction time= void volume/flow rate

FIGURE I

COLUMNS OF SOIL CRUMBS PERFUSED WITH SUBSTRATE: PHOSPHATASE ACTIVITY AND RELATION OF REACTION RATE TO SUBSTRATE CONCENTRATION.

WEIGHT OF CRUMBS: 20 GM

FLOW RATE: 0.901 ML/MIN



flow rates (1,3). The phosphatase activity increased with substrate concentration, reaching an average value of 1.03 micromoles/gm/hr at a concentration of 3.00×10^{-3} M.

For substrate concentrations of 1.00×10^{-4} M to 1.00×10^{-3} M the concentration of product in the column effluents, (P), was proportional to log So/(So-P) indicating the phosphatase reaction in the columns obeyed Michaelis-Menten kinetics (1). For substrate concentrations above 1.00×10^{-3} M the concentration of product in the effluents was higher than that predicted by the kinetics. From the range of substrate concentration in which Michaelis-Menten kinetics were obeyed the value for the constant Vmax was 1.14 micromoles/gm/hr and that for Km was 4.13×10^{-4} M.

B. Hydrolysis of para-nitrophenol phosphate by soil crumb columns of varying lengths

Columns containing 80, 40, and 8.0 grams of soil crumbs were prepared and perfused with substrate in buffer as before. The diameter of the soil columns was 2.1 cm and the heights of the columns were 36, 17.3, and 3.6 cm respectively. The void volumes of the columns were 100, 50, and 10 cm^3. The columns were perfused at a flow rate of 3.05 ml/min. This rate corresponds to a solution velocity of 1.10 cm/min through the soil columns and reaction times of 32.8, 16.4, and 3.28 minutes, respectively. Two columns were perfused for each value of substrate

concentration used. During perfusion the temperature of the columns was maintained at 25.0 °C. The results of the experiment are shown in Figures 2, 3, and 4.

The results show that the phosphatase activity of the columns increased with substrate concentration in a manner similar to that of the previous 20 gram columns. At a substrate concentration of 3.00×10^{-3} M the phosphatase activities of the 80, 40, and 8.0 gram columns were 1.03, 1.10, and 1.13 micromoles/gm/hr, respectively.

For substrate concentrations of 1.00×10^{-4} M to 1.00×10^{-3} M the concentration of product in the column effluents was proportional to log So/(So-P) indicating the phosphatase reaction in the columns obeyed Michaelis-Menten kinetics. At higher substrate concentrations the concentration of product in the effluents was higher than that predicted by the kinetics. From the range of substrate concentration in which Michaelis-Menten kinetics were obeyed, the constant Vmax for the 80, 40, and 8.0 gram columns was calculated to be 1.01, 1.03, and 1.12 micromoles/gm/hr respectively, and the constant Km was calculated to be 3.19×10^{-4} M, 2.87×10^{-4} M, and 3.40×10^{-4} M.

C. <u>Phosphatase activity of soil crumbs in a stationary reaction system</u>

The phosphatase activity of the soil crumbs was determined in a stationary reaction system at various

FIGURE 3

COLUMNS OF SOIL CRUMBS PERFUSED WITH SUBSTRATE: PHOSPHATASE ACTIVITY AND RELATION OF REACTION RATE TO SUBSTRATE CONCENTRATION.

WEIGHT OF CRUMBS: 40 GM

FLOW RATE: 3.05 ML/MIN

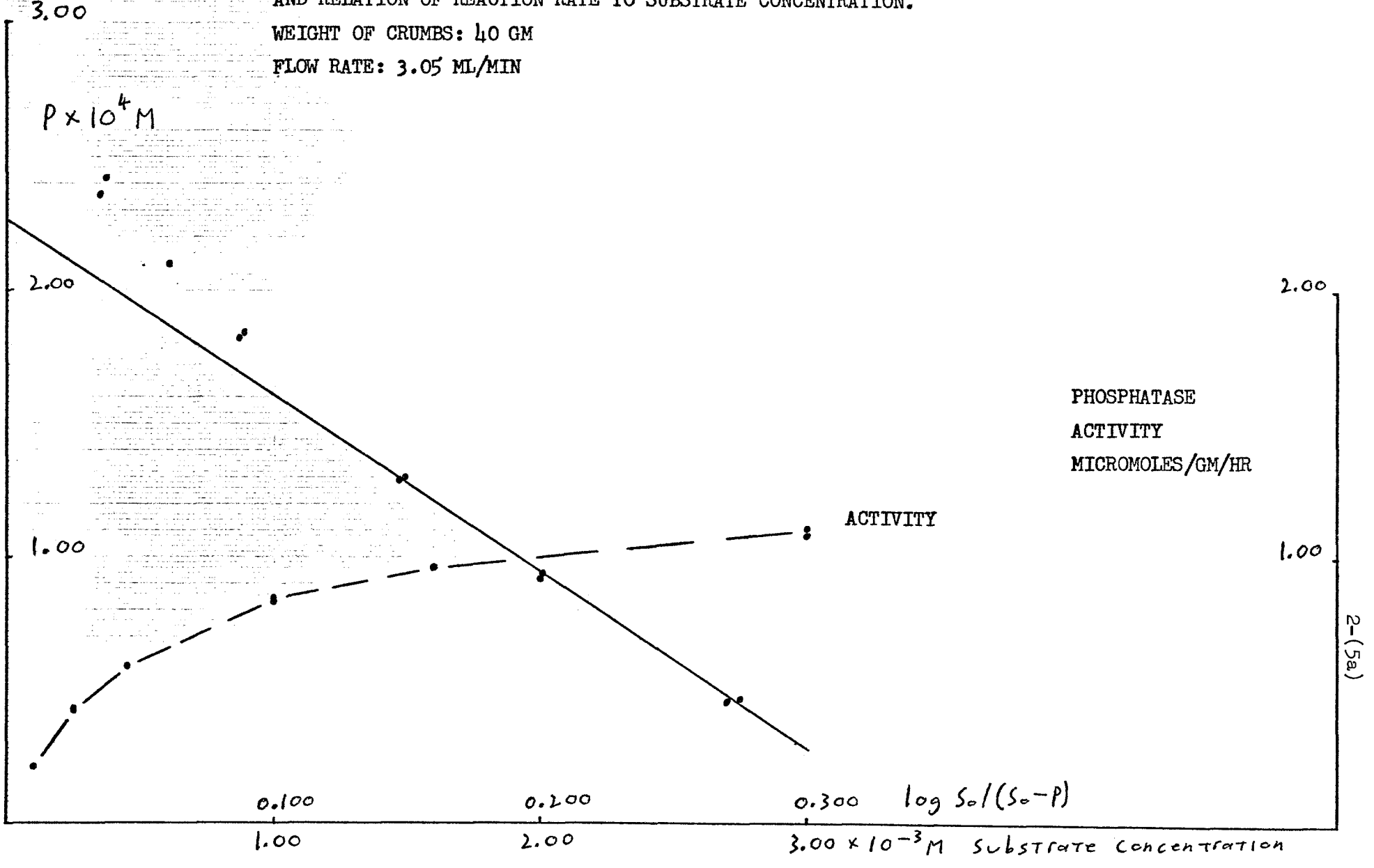
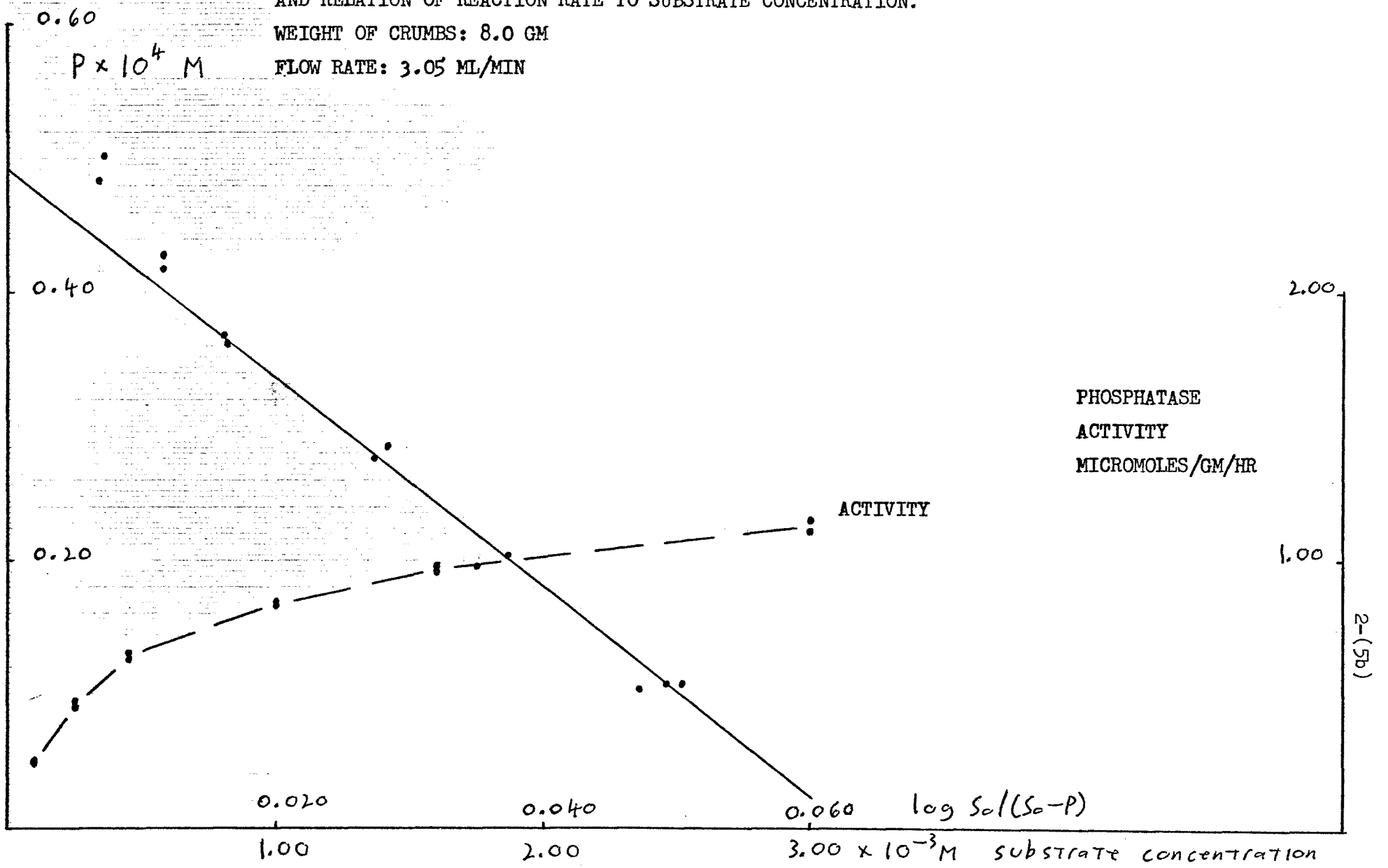


FIGURE 4

COLUMNS OF SOIL CRUMBS PERFUSED WITH SUBSTRATE: PHOSPHATASE ACTIVITY AND RELATION OF REACTION RATE TO SUBSTRATE CONCENTRATION.

WEIGHT OF CRUMBS: 8.0 GM

FLOW RATE: 3.05 ML/MIN



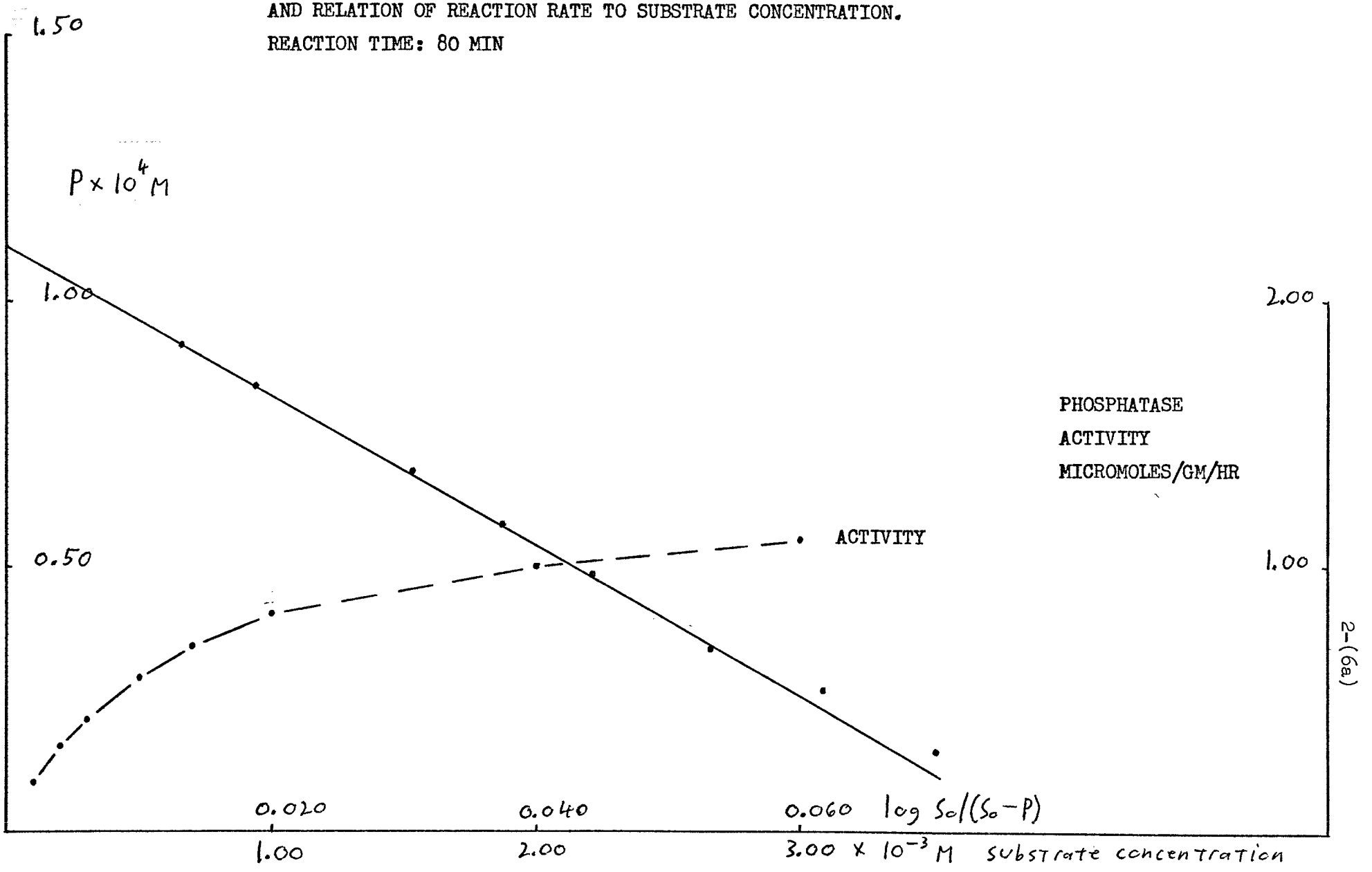
concentrations of substrate. The crumbs were allowed to react with substrate in small flasks which were not agitated during the reaction period. Two determinations of activity were performed: in the first, the reaction time was 80 minutes; in the second, it was 160 minutes. The results of the two experiments are shown in Figures 5 and 6.

The data show that the phosphatase activity of the crumbs in the flask system increased with substrate concentration in a manner similar to that of the crumbs in the columns. At a substrate concentration of 3.00×10^{-3} M the phosphatase activity of the crumbs allowed to react 80 minutes was 1.10 micromoles/gm/hr and the activity of the crumbs allowed to react 160 minutes was 1.06 micromoles/gm/hr.

For both experiments, the concentration of product in the flasks was proportional to log So/(So-P) indicating that the phosphatase reaction catalyzed by the crumbs in the flasks obeyed Michaelis-Menten kinetics. In contrast to the results obtained with the perfused columns, the phosphatase reaction in the flask system obeyed Michaelis-Menten kinetics throughout the entire range of substrate concentration used, 1.00×10^{-4} M to 3.00×10^{-3} M. For the crumbs allowed to react 80 minutes the value of Vmax was 1.32 micromoles/gm/hr and the value of Km was 6.14×10^{-4} M. For the crumbs allowed to react 160 minutes the value of Vmax was 1.30 micromoles/gm/hr

FIGURE 5

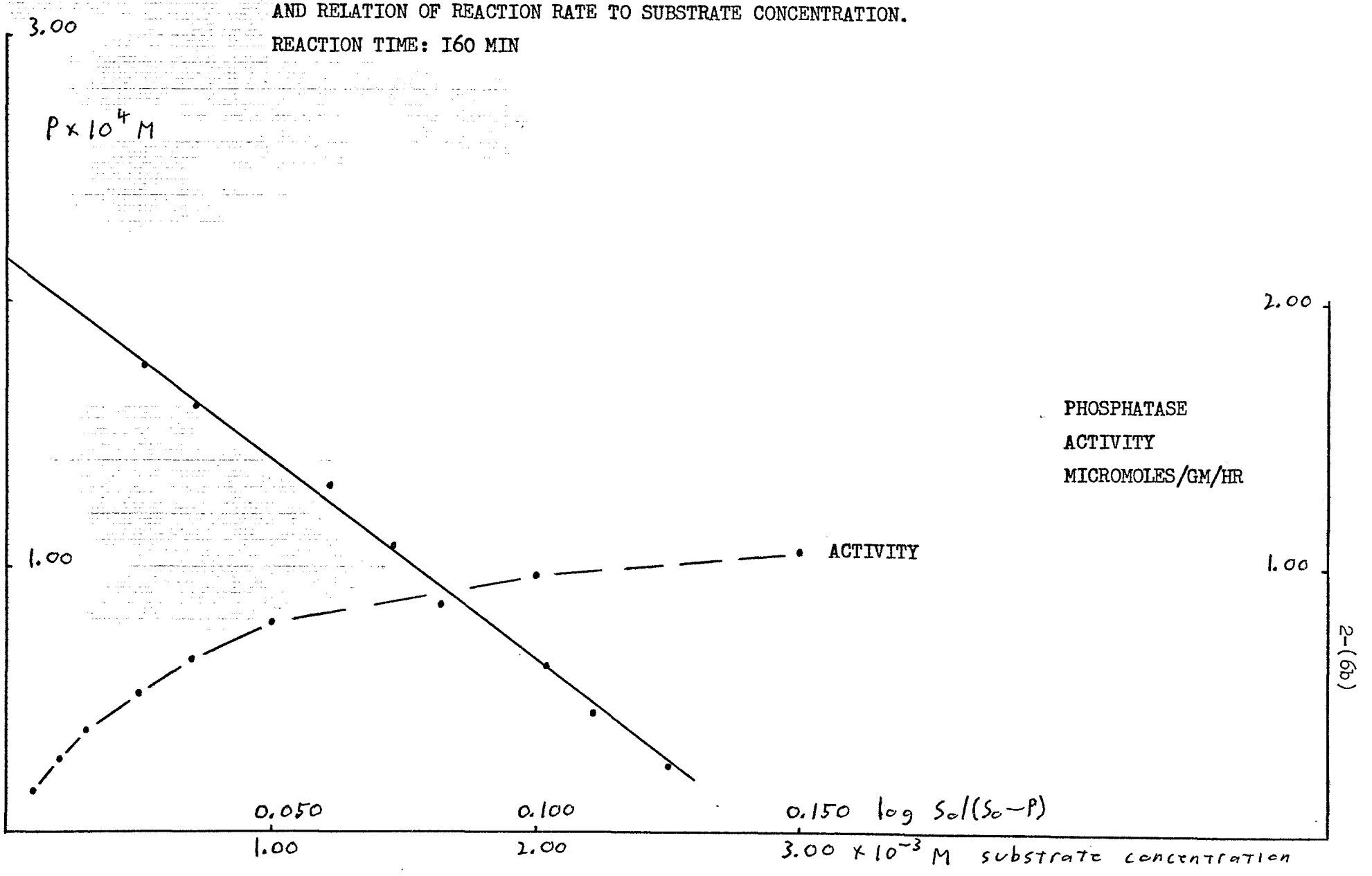
SOIL CRUMBS IN A STATIONARY FLASK REACTION SYSTEM: PHOSPHATASE ACTIVITY AND RELATION OF REACTION RATE TO SUBSTRATE CONCENTRATION.
REACTION TIME: 80 MIN



2-(6a)

FIGURE 6

SOIL CRUMBS IN A STATIONARY FLASK REACTION SYSTEM: PHOSPHATASE ACTIVITY AND RELATION OF REACTION RATE TO SUBSTRATE CONCENTRATION.
REACTION TIME: 160 MIN



and the value of Km was 6.62×10^{-4} M.

D. Phosphatase activity of crushed soil crumbs in suspension

The phosphatase activity of the soil crumbs was determined under conditions in which the crumb material was dispersed to expose all of the material of the crumbs to the substrate solution. The activity was determined following the procedure for determining the activity of soil in suspension, using the crumbs instead of soil (1). Just before adding the substrate the crumbs in the tubes were crushed and stirred with a stirring rod until the crumb material was completely dispersed in the reaction solution. The activity of the resulting suspension was measured at a substrate concentration of 3.00×10^{-3} M. The pH of the reaction mixtures was 6.90. The activity of the crushed crumbs was determined in quadruplicate.

Under these conditions the phosphatase activity of the dispersed crumbs was 1.21 ± 0.01 micromoles/gm/hr.

Discussion

The phosphatase activity of New Dublin soil has been measured with the soil in four different systems: sieved soil in suspension, crushed crumbs in suspension, crumbs in perfused columns, and crumbs in stationary flasks (1,3, and this report). The phosphatase activity was measured at a substrate concentration of 3.00×10^{-3} M. At this concentration the activity of the soil in the different systems is nearly maximal and is relatively insensitive to the small decrease in the substrate concentration that occurs during the reaction. The values for the phosphatase activity of the soil in the different systems are summarized in the table below (p. 9).

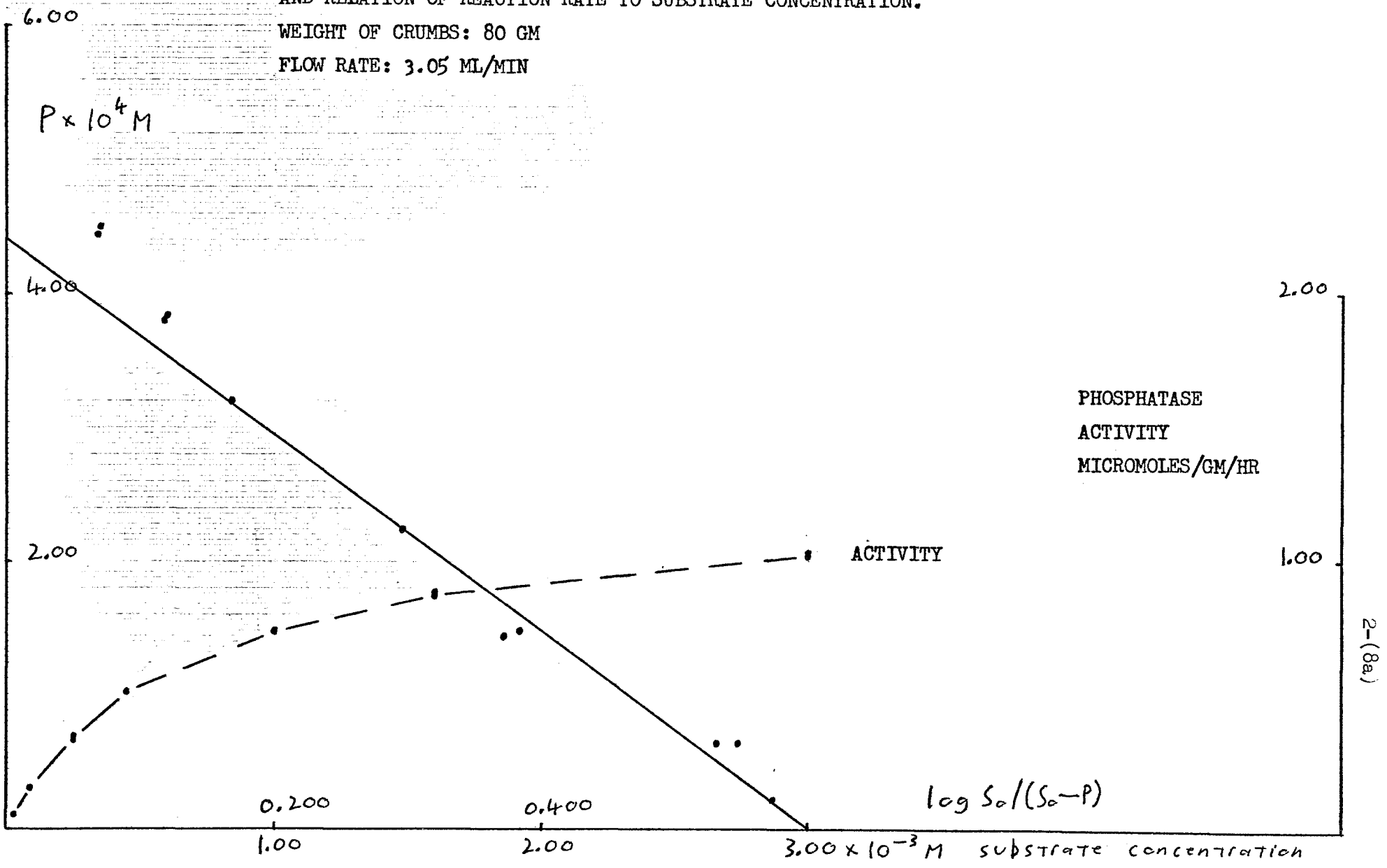
The activity of the sieved soil in suspension was 1.40 micromoles/gm/hr. The activity of the crushed crumbs in suspension was 1.21 micromoles/gm/hr. This value represents the activity of the material of which the crumbs consist. The data indicate that the material in the crumbs has 13.5% less activity than the sieved soil from which the crumbs are made. The lower activity of the crumb material may be the result of inhibition of the phosphatase reaction by the Krilium in the crumbs. Krilium is a synthetic polycarboxylic acid, and it has been found that at a pH value of 6.90

FIGURE 2

COLUMNS OF SOIL CRUMBS PERFUSED WITH SUBSTRATE: PHOSPHATASE ACTIVITY AND RELATION OF REACTION RATE TO SUBSTRATE CONCENTRATION.

WEIGHT OF CRUMBS: 80 GM

FLOW RATE: 3.05 ML/MIN



the phosphatase activity of New Dublin soil is lower in solutions buffered by carboxylic acids than in solutions buffered by amino compounds (3).

sample	phosphatase activity, So = 3.00×10^{-3} M. micromoles/gm/hr.	Vmax micromoles/gm/hr.	Km ($\times 10^{-4}$M)
sieved soil	1.40	1.28	1.60
crushed crumbs	1.21	-	-
20 gm columns:			
Q = 0.901	1.03	1.14	4.13
1.48	1.07	1.12	3.30
2.80	1.12	1.17	3.78
9.46 ml/min	1.21	1.22	3.94
80 gm column Q = 3.05 ml/min	1.03	1.01	3.19
40 gm column Q = 3.05 ml/min	1.10	1.03	2.87
8.0 gm column Q = 3.05 ml/min	1.13	1.12	3.40
stationary flasks	1.10	1.32	6.14
	1.06	1.30	6.62

The values for the phosphatase activity of intact crumbs varied from 1.03 to 1.21 micromoles/gm/hr. These values are 0-15% less than that of the crushed crumbs in suspension. For the columns, no loss of catalytic material occurred during perfusion; no phosphatase

activity could be detected in the effluents of the columns. The data indicate that 85-100% of the catalytic material in the crumbs participates in the phosphatase reaction. The rate of the phosphatase reaction at the interior of the crumbs may be somewhat limited by the rate of diffusion of the substrate to catalytic sites at the interior of the crumbs (2,4).

The phosphatase reaction catalysed by New Dublin soil in the various reaction systems obeyed Michaelis-Menten kinetics. The crumbs in the stationary flask system displayed such kinetics throughout the entire range of substrate concentration used (1.00×10^{-4} M to 3.00×10^{-3} M). However, the sieved soil in suspension and the columns of crumbs displayed such kinetics only with substrate concentrations of 1.00×10^{-4} M to 1.00×10^{-3} M. Above 1.00×10^{-3} M concentration the observed activity was higher than that predicted by the Michaelis-Menten equation. The cause of this difference in behavior is obscure.

The value of the Michaelis-Menten constant Vmax for the sieved soil in suspension was 1.28 micromoles/gm/hr. The values of Vmax for the soil crumbs in the stationary flask system were 1.32 and 1.30 micromoles/gm/hr. The similarity in the values of Vmax suggests that the material in the crumbs actually has about the same phosphatase activity as untreated New Dublin soil and

that the lower values for phosphatase activity of the crumbs compared to the sieved soil in suspension at a substrate concentration of 3.00×10^{-3} M are the result of competitive inhibition of the phosphatase reaction by the Krilium and of a limitation in the rate substrate molecules diffuse to catalytic sites at the interior of the crumbs.

The values of Vmax for the columns of crumbs were 5-21% lower than that of the sieved soil in suspension. It is difficult to draw conclusions from the differences in such values since the environment in which the phosphatase reaction takes place in the crumbs in the columns may be altered by the flow of substrate solution during perfusion.

The value of Km for the sieved soil in suspension was 1.60×10^{-4} M. The values of Km for the crumbs in the stationary flask system were 6.14×10^{-4} M and 6.62×10^{-4} M for the 80 minute and 160 minute reaction times, respectively. The higher values of Km for the crumbs in the flask system may reflect inhibition of the phosphatase reaction by the Krilium and may also reflect a limitation in the rate substrate molecules diffuse to catalytic sites in the interior of the crumbs.

The values of Km for the crumbs in the columns were significantly lower than those for the crumbs in the flask system. It may be that inhibitors present in the crumbs are washed out during perfusion lowering the value of Km from its values in the flask system.

The phosphatase activity of the columns of crumbs and the Michaelis-Menten constants Vmax and Km increased somewhat as the length of the column was decreased and as the flow rate was increased. These effects may arise from differences in the pattern of flow of the substrate solution through the columns or from a gradual decline in the phosphatase activity of the crumbs during perfusion. Samples of the effluent are usually taken for analysis after a volume of solution equal to eight times the void volume of the column has passed through the column. The greater the column length or the lower the flow rate, the longer is the time the crumbs remain immersed in the substrate solution before samples of the effluent are taken for analysis.

The crumbs in the stationary flask system were intended as a model for columns of crumbs perfused at a zero flow rate. Since the values of Vmax and Km for the flask system were significantly higher than those for the columns, this model appears to be inappropriate.

References

1) El-Sayed, M. H., and McLaren, A. D. Enzyme activity in terrestrial soil in relation to the exploration of the Martian surface. The Twelfth Semiannual Progress Report, Space Sciences Laboratory, section III-3, July 1, 1970. University of California, Berkeley.

2) Lilly, M. D., and Sharp, A. K. Chemical Engineer 215, CE12 (1968)

3) McLaren, A. D. Enzyme activity in terrestrial soil in relation to the exploration of the Martian surface. The Thirteenth Semiannual Progress Report, Space Sciences Laboratory, section III-3, January 1, 1971. University of California, Berkeley.

4) Sharp, A. K., Kay, G., and Lilly, M. D. Biotechnology and Bioengineering XI, 363 (1969)